Megazyme

MICROPLATE FORMAT
ASSAY PROCEDURE
For

ALPHA AMYLASE (CERALPHA METHOD)

CERA-MPF
05/09

EQUIPMENT (RECOMMENDED):

1. Disposable 96 well polystyrene clear, flat bottom microplates e.g. Matrix Technologies Corp. cat. no. 4915 (www.matrixtechcorp.com).
2. Disposable 25 mL reagent reservoirs, e.g. Matrix Technologies Corp. cat. no. 8093 (www.matrixtechcorp.com).
3. Multichannel Micro-pipettors, e.g. Gilson Pipetman® Ultra 8-channel (1-20 µL and 20-300 µL).
4. Stop clock.
5. Microplate shaker, e.g. Heidolph Titramax 100 or 1000 (www.heidolph-instruments.com).
6. Microplate reader set at 400 nm.

ASSAY PROCEDURE:

NOTE:

1. Prepare the required reagents and cereal extracts as described in the data booklet for K-CERA, downloadable from where the product appears on the Megazyme website (www.megazyme.com).
2. For each batch of samples that are applied to the determination of α-amylase activity, **a standardised α-amylase control extract must be included on the same microplate**.
3. To compare between the microplate assay (performed at room temperature, ~20°C) and the traditional K-CERA assay (performed at 40°C), the user must perform the standard manual assay procedure (K-CERA) using a standardised α-amylase control extract at both 40°C and room temperature and over the same incubation period as is used in the microplate assay. It is recommended that the control flour extract used here is the same as is used in the microplate assay.

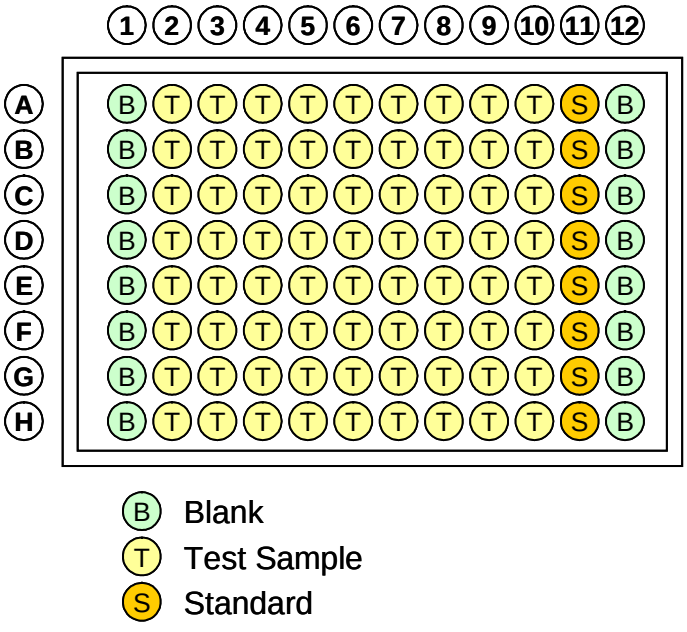
A. Wheat and barley flours:

1. Pour the **Amylase HR Reagent** solution (unbuffered) into a reagent reservoir and dispense **20 µL** aliquots into all wells of the microplate. **Perform this transfer column-wise using a multichannel pipette**. Pre-incubate the microplate and contents at room temperature for 5 min. **This is the assay plate.**
2. Transfer ~100 µL of the individual cereal extract(s) into wells of a second microplate starting at A2 and proceeding, column-wise, no further than H10. (columns 1 and 12 are for reagent blanks; column 11 if for the standardised α-amylase control extract; see **Figure 1** for the layout of the "assay plate"). **This is the cereal extract plate**.
3. Transfer ~100 µL of the standardised α-amylase control extract to each well of column 11 on the cereal extract plate.
4. To start the reaction, transfer **20 µL** of pre-equilibrated wheat or barley extract from the cereal plate directly to the bottom of the corresponding well on the assay plate. **Perform this transfer column-wise using a multichannel pipette at timed intervals** (i.e. transfer column 2 at 0 sec, column 3 at 30 sec, column 4 at 1 min and so on). Shake the microplate vigorously on a plate shaker for ~5 sec after each column transfer and incubate the assay plate at **room temperature** for exactly **forty (40) min** (from the time of addition).
5. At the end of the **40 min** incubation period, add exactly **300 µL** of Stopping Reagent to the wells on the assay plate in the same timed interval sequence as was used to start the reaction. **Perform this transfer column-wise using a multichannel pipette**. Also add exactly **300 µL** of Stopping Reagent to columns 1 and 12 (reagent blanks).
6. Read the microplate at **400 nm**.

B. Malt and microbial preparations:

1. Pour the **Amylase HR Reagent** solution (unbuffered) into a reagent reservoir and dispense **20 µL** aliquots into all wells of the microplate. **Perform this transfer column-wise using a multichannel pipette**. Pre-incubate the microplate and contents at room temperature for 5 min. **This is the assay plate.**
2. Transfer ~100 µL of the individual cereal extract(s) into wells of a second microplate starting at A2 and proceeding, column-wise, no further than H10. (columns 1 and 12 are for reagent blanks; column 11 if for the standardised α-amylase control extract; see **Figure 1** for the layout of the "assay plate"). **This is the cereal extract plate**.
3. Transfer ~100 µL of the standardised α-amylase control extract to each well of column 11 on the cereal extract plate.
4. To start the reaction, transfer **20 µL** of pre-equilibrated malt or microbial extract from the cereal plate directly to the bottom of the corresponding well on the assay plate. **Perform this transfer column-wise using a multichannel pipette at timed intervals** (i.e. transfer column 2 at 0 sec, column 3 at 30 sec, column 4 at 1 min and so on). Shake the microplate vigorously on a plate shaker for ~5 sec after each column transfer and incubate the assay plate at **room temperature** for exactly **twenty (20) min** (from the time of addition).
5. At the end of the **20 min** incubation period, add exactly **300 µL** of Stopping Reagent to the wells on the assay plate in the same timed interval sequence as was used to start the reaction. **Perform this transfer column-wise using a multichannel pipette**. Also add exactly **300 µL** of Stopping Reagent to columns 1 and 12 (reagent blanks).
6. Read the microplate at **400 nm**.

Figure 1 Layout of the "assay plate"



CALCULATION OF ACTIVITY:

One Unit of activity is defined as the amount of enzyme, in the presence of excess thermostable α-glucosidase, required to release one micromole of *p*-nitrophenol from BPNPG7 in one minute under the defined assay conditions, and is termed a **Ceralpha Unit (CU)**.

CALCULATION OF ACTIVITY:

NOTE:

1. Where a standard microplate is employed (i.e. without a path-length correction capability) use the calculations as described below.
2. Where the microplate reader employed has the capability to correct absorbance readings to a 1 cm path-length then the calculations described in the data booklet for K-CERA, downloadable from where the product appears on the Megazyme website (www.megazyme.com), can be used. In this instance these calculations can be simplified by using the Megazyme ***Mega-Calc***™, also downloadable from where the product (K-CERA) appears on the Megazyme website (www.megazyme.com).

Units (U)/g of flour:

$$= \frac{\text{Standard (U)}}{(\Delta A_{\alpha\text{-amylase std}} / T \times F)} \times (\Delta A_{sample} / T \times F)$$

where:

ΔA_{sample} = Absorbance (test) – Absorbance (reagent blank)

$\Delta A_{\alpha\text{-amylase std}}$ = Absorbance (α-amylase standard) – Absorbance (reagent blank)

Standard (U) = Activity of standardised α-amylase control extract (Ceralpha Units)

T = Incubation time (minutes)

F = Dilution factor of original extract

NOTE: Any further dilution of the origin extract must also be accounted for in the calculation.

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